AUTOMATED SPECTROPHOTOMETRIC DETERMINATION OF NITRATE AND NITRITE BY HYDRAZINE REDUCTION IN AQUAEOUS SOLUTIONS AND IN EXTRACTS OF MEAT AND MEAT PRODUCTS

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SUMMARY: Determination of nitrate in pure aquaeous solutions by hydrazine reduction using an automatic analyzer shows that the nitrate $\longrightarrow$ nitrite reduction takes place rapidly but the subsequent reduction of nitrite is much slower. However, in extracts of meat and meat products the second step - the reduction of nitrite - is inhibited. This fact can serve as the basis of exact determination of nitrate in meat products by hydrazine sulphate. Comparison of the generally used cadmium reduction method and hydrazine method shows that the expected values of both methods are the same.

INRTODUCTION: Nitrate determination has always been a much discussed problem in case of simultaneous analysis of nitrate and nitrite in meat products. According to the references, reduction of nitrate to nitrite and then a spectrophotometric determination is the most wide-spread method /Usher et al., 1975/. In Europe, the reduction by cadmium is used where the nitrite - either originally present or formed from nitrate - - is not reduced further. Enzymatic reduction of nitrate seems also to be adequate /Arneth et al., 1988/, but regarding the other reducing agents the opinions are different. Some authors noted /Mullin et al., 1955; Sawicki et al., 1971/ that hydrazine sulphate reduced the nitrite too, while others /Terrey, 1966; Kamphake et al., 1967/ contradicted. Durand /1984/ reported a method of nitrate determination based on hydrazine reduction but only for semi-quantitative evaluation. To make the problem more clear, the reduction of nitrate and nitrite in pure aquaeous solutions and in extracts of meat and meat products /dry sausages/ was studied. Analyses were based on equivalent solutions of nitrate and nitrite. Such aquaeous solutions - strictly under the same conditions - - have shown equal spectrophotometric absorbances after hydrazine reduction and colour reaction /Kamphake et al., 1967/. If equivalent nitrate and nitrite solutions show equal absorbances, then any mixtures of the two equivalent solutions will give exactly the same absorbance:

$$A_{EqNO_3^-red.} = A_{EqNO_2^-red.} = A_{Eq(NO_3^-+NO_2^-)red.}$$

However, strictly the same conditions can not be reached when the determinations are carried out manually. The reproducibility is much higher with an automatic analyzer.

MATERIALS AND METHODS:

Equivalent solutions

For this study the symbol Eq means 0,1 mMol/dm i.e. 0,0101 g KNO_3 or 0,0069 g $NaNO_2$ dissolved in 1 dm^3 distilled water or in 1 dm^3 extract of lean pork meat or dry sausage. Each solution had a pH-value of 8,0.

Extracts of meat and dry sausages

10 g sample /minced meat or dry sausage/ was heated in about 100 cm^3 distilled water at 80 °C for 20 min, with occasional mixing. After cooling, 5 cm^3 Carrez I. and 5 cm^3 Carrez II. solutions were added. The sample was held at room temperature for 30 min, then the pH adjusted to 8,0 with 0,5 M NaOH solution. It was diluted to 250 cm^3 with distilled water and filtered.

Analysis with autoanalyzer

Apparatus: CONTIFLO automatic analyzer /Labor MIM, Hungary/
Reagents:

- sodium hydroxide: 20 g/1 dm^3 distilled water,
- reducing agent : 0,6 g hydrazine sulphate and 0,006 g copper(II.)sulphate pentahydrate/1 dm^3 distilled water,
- acetone : 12,5 % (v/v) in distilled water,
- colour reagent : "A"= 6 g sulphanilic acid and 200 cm^3 conc. acetic acid/1 dm^3 distilled water,
  "B"= 0,30 g alpha naphtylamine and 200 cm^3 conc. acetic acid/1 dm^3 distilled water,
- washing liquor : 0,1 % Brij30 in distilled water.

The flow rate of the reagents and the sample through the autoanalyzer is shown in Figure 1. Speed of the sample is: 60 samples/hour, total flow time of a sample: 10 min.

RESULTS AND DISCUSSION

1./ Reduction in pure aquaeous solutions

Results have proved the previous hypothesis that the mixtures of optional proportions of equivalent nitrate and nitrite solutions show equal absorbances if they are reduced under the same conditions /Figure 2.: dotted horizontal line/. If the colour formation in the $EqNO_2^-$ solution occurs without previous reduction, the absorbance will be higher since the original nitrite content is not decreased by rduction i.e.:

$$A_{EqNO_2^- n.r.} > A_{EqNO_2^- red.}$$

By taking an optional part of the examined $Eq(NO_3^- + NO_2^-)$ series as it is marked with a vertical line in Figure 2.,

the following equation is valid:

$$A_{NO_3^-red.} = A_{(NO_3^-+NO_2^-)red.} - A_{NO_2^-red.}$$

Under the present analytical conditions the ratio of reduced and non-reduced nitrite is constant and is independent of the concentration of nitrite. So, it can be considered as "nitrite factor" i.e.:

$$\frac{A_{NO_2^-red.}}{A_{NO_2^-n.r.}} = f_{NO_2^-}$$

consequently:

$$A_{NO_3^-red.} = A_{(NO_3^-+NO_2^-)red.} - A_{NO_2^-n.r.} \times f_{NO_2^-}.$$

Conclusions: Since the equivalent nitrate + nitrite series in Figure 2. include all possible nitrate/nitrite ratios, it can be established that under the same conditions hydrazine reduction of nitrate and nitrite in aquaeous solutions is independent of their ratio in the tested range. Consequently the nitrate $\longrightarrow$ nitrite reduction takes place rapidly while the subsequent reduction of nitrite is slower and does not depend on whether nitrite is reduced from nitrate or it is present originally. So, from an aquaeous solution with unknown nitrate and nitrite concentrations both components can be determined quantitatively. There is no need for nitrate standards because the ratio of the reduced and non-reduced nitrite is constant.

2./ Reduction in extracts of meat and meat products

The determinations of nitrate and nitrite in extracts obtained as described above, show that the second step, reduction of nitrite does not occur. In present work we did not study which component of the extract inhibits the reduction of nitrite, but it was established that the inhibiting effect can be detected in the extracts up to a dilution ratio of max. 1 : 5.
Analytical results of the extracts are shown in Figure 3. Absorbances of the extracts without added nitrate and nitrite were determined before the experiments and these served as blanks from the appropriate figures. According to the analyses:

$$A_{EqNO_2^-red.} = A_{EqNO_2^-n.r.} = A_{EqNO_3^-red.}$$

namely the concentration of nitrite in extracts of meat and dry sausages does not change through the reduction. However reduction of nitrate to nitrite takes place fully which is verified by $EqNO_3^-$ showing the same absorbance as the non-reduced aquaeous $EqNO_2^-$ solution:

$$A_{EqNO_3^- red.(in\ meat\ extract)} = A_{EqNO_2^- n.r.\ (in\ aquaeous\ solution)}.$$

Therefore concentrations of nitrate and nitrite in extracts of meat and dry sausages can be determined and calculated from the absorbance by means of an aquaeous nitrite standard. However, aquaeous nitrate standard must not be used since in pure aquaeous solutions there is a steady reduction of nitrate and nitrite, so the absorbances can not serve as the basis of calculation.

On the basis of our observations we have carried out determinations of nitrate and nitrite from meat products, by using aquaeous nitrite standards. It was established that the relationship between the absorbances and concentrations is linear in the range: 0,4-5,6 micrograms NO_3^-/cm. The comparison of the hydrazine method with the cadmium method shows, that using the cadmium method as a reference one, the Deming's regression equation furnishes a biasfree estimation (n=52; r=0,9967; $\hat{y} = \hat{x}$) proving that the expected values of both methods are the same.

CONCLUSION: The nitrite reduction by hydrazine sulphate is inhibited in meat extracts. For this reason a simple, rapid, automated spectrophotometric method for nitrate determination from meat products has been elaborated, which can be used instead of the rather complicated and time-consuming cadmium method.

REFERENCES:

- Arneth,W., Herold,B. (1988) Fleischwirtschaft 68: 761.
- Durand,M. (1984) Report of the 13th Meeting of ISO/TC34/SC6.
- Kamphake,L.J., Hannah,S.A., Cohen,J.M. (1967) Water Res. 1: 205.
- Mullin,J.B., Riley,J.P. (1955) Anal.Chim.Acta 12: 464.
- Sawicki,C.R., Scaringelli,F.P. (1971) Microchem J. 16: 657.
- Terrey,D.R. (1966) Anal.Chim.Acta 34: 41.
- Usher,C.D., Telling,G.M. (1975) J.Sci.Fd.Agric. 2: 1793.

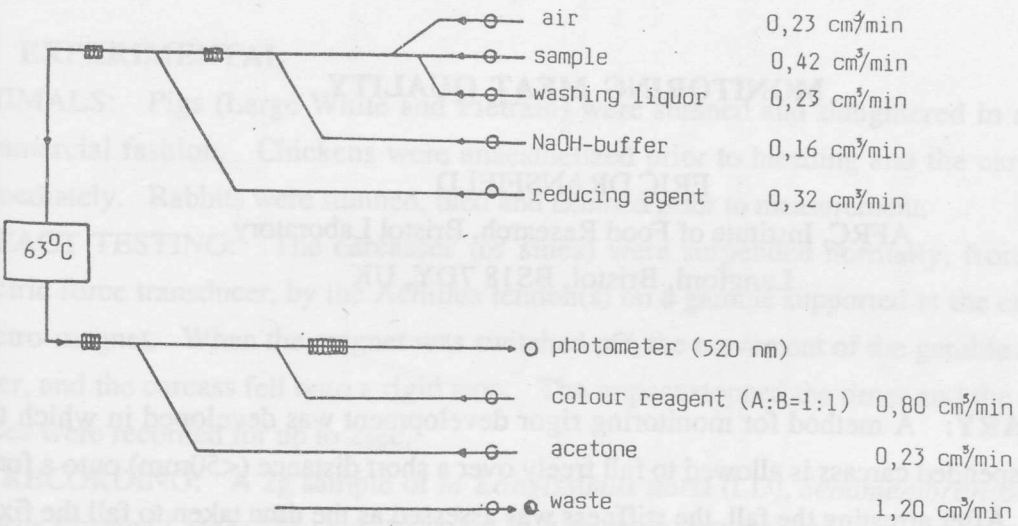

Figure 1. Flow diagram for nitrate/nitrite determination by hydrazine reduction

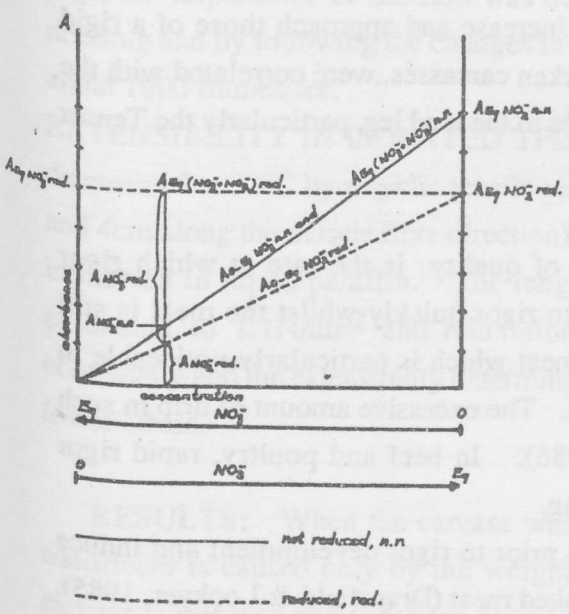

Figure 2. Reduction of nitrate and nitrite by hydrazine sulphate in pure aquaeous solutions

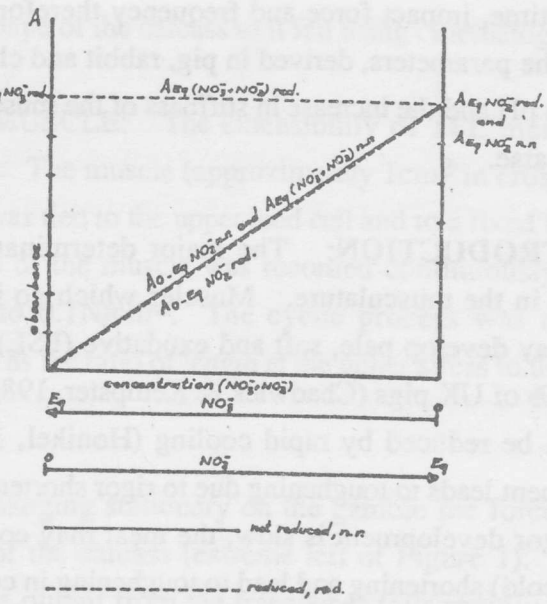

Figure 3. Reduction of nitrate and nitrite by hydrazine sulphate in extracts of meat and meat products